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# Advances in Soil Amendment: Vermicomposting, Alumina Refinery Residue and Cotton Production in Australia

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Abstract— Cotton production is an important primary industry in Australia. However, the industry faces several challenges, including access to fresh water, disposal of cotton waste, and the ability of Australian soil to sustain pesticide-intensive cultivation. Research programs into a variety of techniques aimed at amending soil with fertilisers, particularly those produced from novel organic sources rather than industrial fertilisers, have also been developed. This paper therefore considered the relationship of worm castings produced from a municipal vermicomposting facility and a modified form of alumina refinery residue (MARR) as a possible nutrient source for cotton farming, and examined their combined potential as a biological fertiliser to amend agricultural soil and improve cotton yields. The study found the addition of MARR enhanced the chemical and biological properties of worm castings, for example improving caution exchange capacity and increasing the total microbial biomass of castings by 38%, and the combined effect of applying 240 L/ha of this non-traditional organic fertiliser increased cotton yields by 9% over a nine-week growing period.

Keywords— Cotton farming, vermicomposting, worm castings, alumina refinery residue, soil amendment.

#### I. INTRODUCTION

The cotton industry is a major contributor to the economic wealth of some countries, including Australia (de Garis, 2013). In fact, more than 100 countries grow cotton commercially, with China (33 million bales/year) and India (27 million bales/year) among the biggest producers; Australia is the third largest exporter of cotton after the U.S. and India, supplying 3% of the world's cotton. Australia also has the highest yields in the world (with highs of 10-12 bales/ha or about 2.1 t/ha) and exported an average of 4.3 million bales in 2012-2103 valued at \$2.7 billion (CSIRO, 2015; Global Agricultural Information Network, 2013).

However, in addition to problems associated with salinity and soil erosion, the over-abundant use of pesticides (including herbicides, insecticides and fungicides) and their downstream impacts on the environment (a global problem identified in earlier studies, such as those of Banuri [1999] in Pakistan), and the limited access to (and hence availability of) enough fresh water for viable production, one of the most pressing issues faced by the cotton industry is the capability of farmland to sustainability support long-term production. As a consequence, the need for a feasible and economically viable approach to soil amendment as been encouraged.

The primary waste stream generated from cotton (*Gossypium hirsutum*) production is cotton gin trash (CGT), the rejected leaves, sticks, bolls and soil which remain after ginning. Disposing CGT as a solid waste (via either incineration or as a solid to landfill) is an option, but Knox *et al.* (2006) have also identified the ways CGT can be reused by the industry, which include spreading untreated CGT on soil to amend it, feeding it to livestock as a hay replacement, and composting it to produce fertiliser. Applying CGT as a soil additive at a rate of 6-15 t/ha has been shown to increase cotton yields, apparently due to increase moisture retention in soil (Knox *et al.*, 2006), and this approach has been considered the most desirable.

In some jurisdictions CGT has been classified as "hazardous" due to the presence of high concentrations of pesticides, particularly chlorpyrifos, chlorfurazuron and endosulfan which can potentially uptake into the cotton stalk (Crossan & Kennedy, 2006), thereby making either spreading CGT untreated on soil or using it as a feed source less attractive. In fact, downstream concerns associated with CGT feed being contaminated with residues of chlorfurazuron and endosulfan (although 56 chemicals associated with pesticide use in cotton production have been isolated) and their potential to find their way into the human food-chain, have been identified (Crossan *et al.*, 2006).

For these reasons, feeding CGT to livestock has been ruled out entirely in Australia, and incineration is discouraged because of its atmospheric polluting and greenhouse gas potentials (i.e., as a result of direct, on-site burning). On- and off-site disposal of CGT as a solid waste is an option, but off-site disposal can be costly and is the least economically desirable approach. Therefore, amending soil by reusing CGT as a media for composting has been considered.

During the last 40 years composting CGT has been investigated, but with mixed results. For example, Gordon *et al.* (2006) found that while CGT produced a viable compost material, the composting process did not destroy either verticullium wilt (a disease caused by soil-borne fungi *Verticillium albo-atrum* and *Verticillium dahliae*) or miscellaneous weeds, including annual bluegrass (*Poa annua*), large crabgrass (*Digitaria sanguinalis*), purple nutsedge (*Cyperus rotundas*) and prickly sida (*Sida spinose*), all associated with "gin trash". On the other hand, Griffis and Mote (1978a, 1978b) found composting CGT was lethal to all weeds present in the gin waste, including redroot pigweed (*Amaranthus retroflexus*) and Johnson grass (*Sorghum halepense*), and the process produced a viable compost.

Similarly, using static aeration biopiles and standard turned windrows, Díaz *et al.* (2002) found that when 55% CGT was mixed with 45% vinasse (obtained by fermenting molasses, sugarcane, maize or beetroot) by weight, peak temperatures of 45°C at 21 days in biopiles and 54°C at seven days in windrows were achieved, and both methods achieved stability and fertiliser of high value. Smith (2009) also found that when using an aerating composter temperatures rose to 55-68°C within 72 hours, thereby killing pathogens and the germination of any seeds; Smith showed he was able to produce 15m<sup>3</sup> of high-grade compost every 24 hours using CGT as a carbon source via this method.

Further areas of research relevant to soil amendment and cotton production are the related fields of vermiculture or "worm farming" (Edwards, 2004; Edwards *et al.*, 2010) and vermicomposting (Dickerson, 2001; Monroe, 2014). Vermiculture is the process whereby any one of 1,800 species of earthworms (usually red earthworms *Eisenia Fetida* or tiger earthworms *Eisenia Jetida*) are cultivated or artificially reared for use in any number of applications, including as bait; vermicomposting is the process whereby the worms used in vermiculture produce "worm castings" or vermicasts (i.e., the waste by-product from digesting organic matter, such as household waste or manure).

Vermicomposting can also be defined as the process whereby worms are actually fed compost in order to generate worm castings. In both cases, worm castings have nutritional value and can therefore be used as a "natural" organic fertiliser in agriculture. The following percentage components of worm castings provides a summary of the chemical properties of "average" worm castings: organic carbon (OC) = 20-30%; nitrogen (N) = 1.8-2.0%; phosphorus (P) = 1.3-1.9%; potassium (K) = 1.3-1.5%; calcium (Ca) = 3.0-4.5%; and <0.5% traces of magnesium (Mg), sodium (Na), sulphur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B) and aluminium (Al) (Bourdon, 2011).

However, vermicomposting can concentrate heavy metals in worm castings if metals are present in the original composted material (e.g., zinc can increase by 70% from 14.5 mg/kg to 24.6 mg/kg as a result of vermicomposting), but more importantly can also increase the percentage of desirable macronutrients. For example, vermicomposting can increase N from 0.5% to 1.6% and P from 0.2% to 0.7% in worm castings when using animal manure as the feedstock, and N from 0.8% to 1.9%, P from 0.35% to 0.47%, K from 0.48% to 0.7%, and Ca from 2.3% to 4.4% when using garden waste as the feedstock.

In the context of flowering pot plant production, Matta *et al.* (2008) have reported that the nutrient value of worm castings generated from the vermicomposting of sheep and cattle manure is greater than those derived from vermicomposting horse manure; Matta *et al.* have also reported that earthworms have a preference for animal manure generally over other types of organic wastes. Significant potential value for soil amendments, including amending soil for cotton production, thereby inheres to worm castings produced through vermicomposting (Dickerson, 2001, p. 1).

Of interest to the present study are the trials of John Buckerfield and his team, whose early work using worm castings mixed with composted CGT as a soil amendment agent to improve cotton production is notable. Buckerfield (1999) found that various addition rates of composted CGT and manure, along with worm castings, increased cotton yields by 15% in the first year after application. Mysteriously, he also found that yields went down by 5% and 25% respectively in the two subsequent years, although he could not attribute these declines to the quality of soil amendment additives. This phenomenon, along with enhanced moisture retention was also investigated in vineyards (Buckerfield *et al.*, 1999) with similar outcomes.

Alumina refinery residue (ARR) or "red mud" has similarly been investigated over several decades as a possible means of amending agricultural soil. For example, in both its unmodified (i.e., high alkalinity >3,000 mg/kg and pH >12.5) and modified (i.e., alkalinity of <300 mg/kg and pH <10.0) forms, research conducted in many countries since the 1980s has been based on a supposition that ARR might help agricultural soil retain moisture, neutralize acidity, bind heavy metals, and enhance the presence, retention and availability of macronutrients (such as Fe) and micronutrients (such as Mn) in soil.

ARR is typically composed of Fe (30-60%), Al (10-20%), Na (3-10%), titanium (4-18%), silica (3-50%) and Ca (2-8%) in oxide, hydroxide and/or oxy-hydroxide states. ARR is composed of a complex of metals and minerals, including hematite (Fe<sub>2</sub>O<sub>3</sub>), beohmite ( $\gamma$ -AlOOH), gibbsite (Al[OH]<sub>3</sub>), sodalite (Na<sub>4</sub>Al<sub>3</sub>Si<sub>3</sub>O<sub>12</sub>Cl), anatase (TiO<sub>2</sub>), aragonite (CaCO<sub>3</sub>), brucite

 $(Mg[OH]_2)$ , diaspore ( $\beta$ -Al<sub>2</sub>O<sub>3</sub>.H<sub>2</sub>O), ferrihydrite (Fe<sub>5</sub>O<sub>7</sub>[OH].4H<sub>2</sub>O), gypsum (CaSO<sub>4</sub>.2H<sub>2</sub>O), hydrocalumite (Ca<sub>2</sub>Al[OH]<sub>7</sub>.3H<sub>2</sub>O), hydrotalcite (Mg<sub>6</sub>Al<sub>2</sub>CO<sub>3</sub>[OH]<sub>16</sub>.4H<sub>2</sub>O) and para-aluminohydrocalcite (CaAl<sub>2</sub>[CO<sub>3</sub>]<sub>2</sub>[OH]<sub>4</sub>.3H<sub>2</sub>O). As Caand silicon (Si) play a vital role in plant nutrition, disease prevention and nitrogen absorption efficiency, the existence of these elements may play a useful role in soil amendment.

However, ARR may contain heavy metals and metalloids, including arsenic (As), chromium (Cr), Cu, gallium (Ga), Mn, thorium (Th), uranium (U) and vanadium, although these are usually only present in trace concentrations of a few parts per million up to 100 mg/kg. While the potential presence of radio nuclides, such as lead (Pb), Th and U, have also raised ongoing health and environmental concerns, according to Gräffe *et al.* (2009) these elements are almost always found in non-radioactive states and in especially low concentrations.

About 50% of ARR is amorphous, with crystalline constituents composed mainly of goethite and hematite, quartz, and rutile, anatase and/or ilmenite; many minor remnant phases from the original bauxite (e.g., mica and boehmite) and newly formed species (e.g., natroalunite and noselite) may be present. Upon contact with water, ARR imparts a pH of  $\pm 12.5$ , with elevated levels of electrical conductivity(EC) at 1.0-16 dS/m due to high concentrations of Na; >80% of ARR particles are <10 micron and thus the media has a high charge-to-mass ratio and a high surface area-to-mass ratio (about 300 mm<sup>2</sup>/g), a bulk density of 1.53 g/cm<sup>3</sup>, anda specific gravity of 3.2g/cm<sup>3</sup> (all data on ARR from Deelwal *et al.*, 2014; Fergusson, 2009, 2015; Gräffe *et al.*, 2009).

According to Snars *et al.* (2003), the global research effort into ARR has concentrated on its potential effects in a number agricultural contexts, including P-retention of sandy soils to prevent phosphate ( $PO_4$ ) run-off to rivers and estuaries (Summers *et al.* 1996a; Summers and Pech, 1997), the so-called "liming" of acidic soils (Simons, 1984), and importantly for this study, increasing yields of horticultural crops and pastures with and without the addition of N, P and K fertilisers (Summer *et al.*, 1996b; Summers *et al.*, 2001).

Research has also been conducted on the use of ARR to beneficiate compost and the composting degradation process. Waddell *et al.* (2002) investigated the impact of ARR on retaining soluble P in compost added to sandy soils and its impact on the growth of long scarlet radishes (*Raphanus sativas*). Starting concentrations of 0.83% total P in compost were detected, achieved as a result of ARR addition rates between 1-5% (on a w/w basis) to sandy soils. Results indicated that radish yields from crops grown in sandy soils amended with ARR-compost were significantly higher than a control soil with and without unamended compost, but yields were not as high as crops grown in sandy soils amended with composts containing other clay-like additives, including zeolite. However, Waddell *et al.* reported a decreased loss of P in soil and a greater retention of P in radish roots when ARR addition rates increased, with P concentrations of 0.38% in roots and 0.24% in leaves 11 weeks after application; Waddell *et al.* also found that ARR significantly aided the retention of N in sandy soils.

Thiyagarajan *et al.* (2011) similarly studied to role of ARR and compost and their relation to Mn-retention in sandy soils, and Anderson *et al.*(2011) investigated whether amending ARR sand (i.e., the courses and fraction of ARR, called the desilication product or DSP present at concentrations of between 3% and 50%) with ARR fines (i.e., the fine sand fraction which is present at concentrations of between 1% and 5%) would improve its suitability as a growth medium. Anderson *et al.* found that the addition of ARR "fines" more than doubled plant-available water and increased extractable nutrient concentrations relative to unaltered sand, and increased extractable K, P, S, Mg and B in the growth medium. However, ARR treatments also increased both the electrical conductivity and exchangeable Na percentage of the growth medium.

This author has reported on the use of a modified form of ARR in both composting and agriculture. Fergusson (2009) documented the application of modified alumina refinery residue (MARR), i.e., ARR which had been modified using seawater and/or the addition of Mg and Ca salts to reduce total alkalinity caused by high concentrations of Na and chloride (Cl) in the original ARR, in a biosolids composting trial at a municipal council site in Queensland. In that study, composting time to stability of biosolids and green waste was reduced from a standard 11-14 week composting period to seven weeks, the amount of carbonaceous green waste required for compost to reach maturity was reduced by 50%, and average biopile temperatures averaged 65°C over seven weeks.

The composting process generally follows three basic phases: first, a mesophilic or "moderate temperature" phase, which lasts between a few days and a week as the temperature rises above 40°C; second, a thermophilic, or "high temperature" phase, which can last for a few days up to a few months at temperatures above 50°C; and third, a "cooling" or maturation phase when temperatures return to 40-45°C. Each phase is dominated by mesophilic, then thermophilic, and then again mesophilic microorganisms, including actinomycetes and saprophytes.

The finding that MARR increases average biopile temperatures to an average of 65°C over seven weeks may not be entirely desirable, as target average temperatures for compost are usually 50°C, with a minimum of only three consecutive days required at temperatures greater than 55°C (although some jurisdictions require temperatures above 65°C for at least three days). Thus, the higher temperatures observed when using MARR in compost may potentially destroy the very beneficial and desirable mesophilic bacteria present during the cooling phase.

However, this author has found no evidence in the literature pertaining to the relationship between vermicomposting and MARR or to the relationship between vermicomposting, MARR and cotton production. This shortfall in data begs the question of whether the combination of vermicomposting and MARR might play a role in cotton production, leading to the present study which asks the following research questions: 1) What effect does the addition of MARR have on the chemical and biological properties of worm castings; and 2) Does the application of a combination of worm castings generated from the composting of municipal household waste and MARR improve cotton yields?

#### II. MATERIAL AND METHOD

New South Wales (NSW) is home to one of the largest vermiculture and vermicomposting waste treatment facilities in the southern hemisphere, where 20 million worms digest hundreds of tonnes of municipal solid household waste (i.e., co-mingled putrescible kerbside and green wastes) each year (Department of Environment and Conservation NSW, 2007; Lismore City Council, 2011). Output from this activity is the annual production of solids which are solubilised and sold as high-grade fertilisers, mostly to the macadamia nut, dairy and cotton industries in in consultation with the NSW Department of Agriculture, although McLean (2004) also reports their use in state forests and viticulture.

The vermicomposting company had experimented with the manufacture of fertilisers from worm castings, but found the 1,000 L plastic intermediate bulk containers (IBCs) in which the castings had been solubilised expanded to the point of almost bursting due to oxidizing effects of decomposing organic matter. The company also wished to enhance the chemical and physical properties of their worm castings in preparation to test and commercialise a range of fertiliser products based on worm castings, and to this end, two laboratory tests were conducted to investigate the impact of MARR on worm castings, and a field trial was carried out to explore the application of a worm castings-MARR blend on cotton production in NSW.

#### 2.1 Phase One

The laboratory tests were conducted to study the effects of MARR addition on worm castings. Modest additions of MARR were expected to enhance soil chemistry and microbial reactions which might prove significant when the resultant fertiliser was applied in close proximity to seed or root systems (i.e., the rhizobium) in hostile, low pH environments and where toxicity due to high soil concentrations of heavy metals reduce plant growth.

The first laboratory test applied the following method: 1) a 270 g sample of oven dried worm castings was tumbled for 24 hours with 540 ml of Milli-Q "ultrapure" water; 2) after tumbling, the sample was allowed to settle for four hours and then dewatered; 3) a 100g sample of settled worm castings was collected and stored for later analysis; 4) a 100 ml of dewatered supernatant liquor (SNL) collected from the top of the sample was collected and stored for later analysis; 5) a 90 g sample of worm castings was mixed thoroughly with 10 g of MARR to form a 100 g sub-sample; 7) the sub-sample was allowed to stand for 24 hours; and 8) the untreated worm castings (step 3), SNL (step 4), and sub-sample (step 7) were submitted to ALS in Brisbane for analysis using relevant instrumentation, including inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-optical emission spectrometry (ICP-OES).

Both the untreated worm castings (step 3) and the sub-sample (step 7) were subjected to the following analysis: exchangeable cations Ca, K, Mg, and Na (measured in centimoles of positive charge per kilogram of soil, i.e., cmol<sup>+</sup>/kg, a measure which has mostly replaced in soil science the earlier measurement of meq/100 g); total metals As, Cd, Cr, copper (Cu), Fe, mercury (Hg), Mn, nickel (Ni), Pb, selenium (Se) and zinc (Zn) in mg/kg; leachable metals in mg/L using the U.S. Environmental Protection Agency's Toxicity Characteristic Leaching Procedure or TCLP (ALS Global, 2011); and leachable metals in mg/L using distilled water (i.e., a "water leach" test). The SNL (removed in step 3) was analysed for total metals in mg/L.

In the second laboratory test, two fertiliser batches of worm castings were prepared according to the following protocol: 1) create a 1,000 L mix of worm castings and fresh water on a 1:1.6 v/v basis (i.e., to produce "compost tea") and store in an IBC; 2) create a 1,000 L mix of worm castings and fresh water on a 1:1.6 v/v basis and then thoroughly mix this compost tea with 10% (i.e., about 100 kg) of dry powdered Viro Grow reagent (moisture content  $\pm 17\%$ ), a v/v mixture of 90% MARR and 10% calcium carbonate [CaCO<sub>3</sub>]) and store in an IBC; 3) aerate both fertiliser batches for 84 hours; and 4) take one 100g

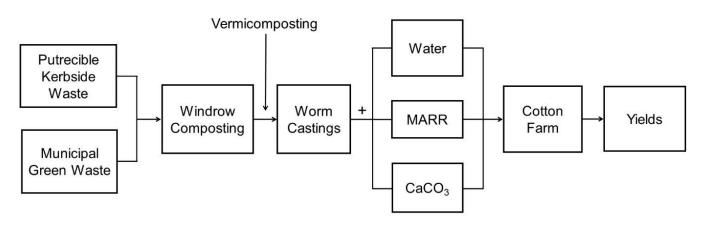
sample from each batch of fertiliser (described in steps 1 and 2) and 5) submit each sample to Soil Foodweb Institute in Lismore for analysis of pH and microbial biomass, specifically measuring total bacteria, total active bacteria, total fungi, and total active fungi, including analysis for pseudomonas, actinomycetes, protozoa, and mycorrhizal fungi, among other microorganisms.

For the purposes of this study, microbial biomass of liquid worm castings was defined as that part of the castings that constitutes living microorganisms smaller than 5.0  $\mu$ m<sup>3</sup>. In agriculture and soil science, microbial biomass is generally expressed in milligrams of carbon per kilogram of soil (mg/kg) or micrograms of carbon per gram of dry weight of soil ( $\mu$ g/g), however in this study microbial biomass was measured as micrograms of carbon per millilitre ( $\mu$ g/ml) of liquid fertiliser.

#### 2.2 Phase Two

A nine-week field trial was conducted at a cotton farm in Narromine, NSW, a part of Australia which has been cropped intensively with cotton for the past 20 years. The intention was to add ViroGrow to two worm castings-based fertilisers, hereafter referred to as Fertiliser #1 and Fertiliser #2. The two fertilisers were exactly the same (i.e., every 1,000 L of fertiliser was a blend of worm castings and fresh water on a 1:1.6 v/v basis mixed with 10% ViroGrow reagent) except Fertiliser #2, which had an additional 5% CaCO<sub>3</sub> mixed on a v/v basis. Both so-called "liquid inoculums" were composed of liquefied worm castings of high organic carbon origin (i.e., humus) derived from the vermicomposting process.

The intention was to produce a blend of phyto-hormones, plant available nutrients, humic and fulvic acid, and a diverse population of beneficial bacteria, fungi, protozoa and nematodes. McLean (2004) describes these collectively as "microbial soil bio-stimulants and growth solutions derived primarily from floral botanicals". Thus, Figure 1, represents the process flow of Phase Two.



#### FIGURE 1: PROCESS FLOW OF PHASE TWO.

The aforementioned "fineness" of MARR, it was planned, would expose a large surface area of Ca- and Si-based minerals which together would allow fungal oxalate secretions to solubilise mineral Ca to form calcium oxalate crystals (e.g.,  $CaC_2O_4$ ). These crystals would be retained in the fungal hyphal mats where they would be resistant to leaching but become available to plants through a symbiotic relationship with soil fungi (a similar phenomenon was earlier described by Giffiths *et al.* [1991] in the context of forestry and Douglas-fir seedlings).

It was hypothesised that when applied as a soil spray (or drench), both Fertilisers #1 and #2 would have an ability to neutralise acidity (due to the acid neutralising capacity of MARR) and, should they be present, reduce toxic levels of heavy metals—including copper, manganese and aluminium (due to the metal binding capacity of MARR)—in the root zone. Whether applied as a spray or drench, it was proposed these fertilisers would prevent root disease organisms and reduce pesticide use, improve nutrient retention (potentially reducing contamination of surface and groundwater), improve nutrient cycling by reducing the need for further fertiliser applications, allow degradation of toxic materials in the soil by sequestering heavy metals and by degrading residues and pesticides, and build soil aggregate structure by improving water holding capacity and diffusing oxygen into soil, thereby reducing disease and improving soil compaction. While these potential micro-benefits were not assessed specifically in this Phase Two of the research, their presence would be inferred if crop yields increased.

A 7.0 ha experimental field, which consisted of a red loamy soil known locally as a "red brown earth" with a pH of 6.5, was used in Phase Two. The experimental field was not considered the best performing on the farm, but consistently yielded above expectation. However, the previous year's crop was patchy throughout the field: the western edge was higher yielding, but the eastern edge was lower yielding as a result of watering difficulties due to unseasonably hotter weather and limited water availability. The experimental field was managed the same as the rest of the farm, and received eight irrigations during the trial period, averaging about 9.0ML/ha. Insect pressure was intense in the early part of the growing season but slowed toward the middle and end of the season, and the number of fungicide, herbicide, insecticide sprays were lower than the average year. The entire field received 230 kg/ha of N, 33 kg/ha of P, and 30 kg/ha of K prior to the experimental period; two foliar applications of multi-nutrient mixtures with an emphasis on K were also applied.

The trial was conducted on three separate 1.32 ha cotton paddocks (each paddock 24 m W x 550 m L) for a total of 3.96 ha within the experimental field. All three paddocks were sown in late December and harvested in mid-March with a standard cotton picker; all 3.96 ha were ginned and assessed after Week 9. Monitoring the results occurred four times over a nine-week growing period, prior to commencement of the trial (Week 0), after three weeks (Week 3), after six weeks (Week 6), and after nine weeks (Week 9) at defoliation. Paddock #1 was treated as a control and received standard fertiliser spray applications; Paddocks #2 and #3 each received spray applications of 40 L/ha (240 L of fertiliser/ha after dilution) respectively of Fertilisers #1 and #2.

Phase Two assessed the following parameters between the time of initial planting and application of fertilisers and nine weeks later at defoliation: 1) height of cotton plants at weeks 0, 3, 6 and 9; 2) the number of cotton nodes (i.e., the number of connections where main stem leaves and branches attach to the plant stem) at weeks 0, 3, 6 and 9; 3) the height-to-node ratio (i.e., height: number of nodes) at weeks 0, 3, 6 and 9; 4) fruiting factor (i.e., the average number of fruiting branches or *sympodia* on a plant) at weeks 0 and 9; 5) nodes above white fruit (i.e., nodes above bolls, a sign of cotton maturity, as described by Bourland *et al.*, 2001); and 6) paddock yields in bales/ha.

## III. **RESULTS**

#### 3.1 Phase One:

Three types of cation exist in soil: unavailable, slowly available, and readily available or "exchangeable" (Rehm & Schmitt, 2002). While total K, for example, can be up to 40,000 mg/kg in soil (i.e., 4% by volume), most K is part of the structural component of soil and is therefore unavailable for plant growth. However, some aspects of K will either be slowly available or readily available as plant nutrition, depending on soil type, soil conditions and the types of plants which phyto-remediate it.

Parameter	Exchangeable Ca (cmol <sup>+</sup> /kg)	Exchangeable K (cmol <sup>+</sup> /kg)	Exchangeable Mg (cmol <sup>+</sup> /kg)	Exchangeable Na (cmol <sup>+</sup> /kg)		
Worm Castings	32	6.7	10.2	2.2		
Sub-sample	37	9.0	12	9.8		

 TABLE 1

 Exchangeable cations in untreated worm castings and in a blend of worm castings and MARR from the first laboratory test in Phase One.

Table 1 presents the results of the first laboratory test related to readily available or exchangeable cations, i.e., cations which are water soluble and bio available, sometimes referred to as the cation exchange capacity (CEC) of soil. Concentrations above 10 cmol<sup>+</sup>/kg are preferred for most plant production, depending on the crop type. [Note, cmol<sup>+</sup>/kg refers to centimoles of positive charged cation per kilogram of soil; in soil science, exchangeable cations are mostly measured in cmol<sup>+</sup>/kg, a parameter which has replaced the early measurement of meq/100 g].

From this data it is clear that worm castings, both before and after the addition of MARR, contain exchangeable cations, and minor changes in exchangeable cations were observed when MARR was added to worm castings: Ca increased from 32  $\text{cmol}^+/\text{kg}$  to 37  $\text{cmol}^+/\text{kg}$ ; K increased from 6.7  $\text{cmol}^+/\text{kg}$  to 9.0  $\text{cmol}^+/\text{kg}$ ; Mg increased from 10.2  $\text{cmol}^+/\text{kg}$  to 12  $\text{cmol}^+/\text{kg}$ ;

and Na increased from 2.2 cmol<sup>+</sup>/kg to 6.7 cmol<sup>+</sup>/kg. The larger increases in K (35%) and Na (350%) are accounted for by the fact that MARR contains an abundance of cations, particularly Na. The increased availability of Na after MARR addition is consistent with the findings of Anderson *et al.* (2011).

In percentage terms, Ca = 50%, K = 10%, Mg = 18% and Na = 10% of total CEC in this finding. Given the desirable ranges for representative exchangeable cations in CEC are Ca = 65-80%, K = 1-5%, Mg = 10-15%, and N = 0-1% of CEC, the balance of cations in both untreated and treated worm castings is low for Ca, roughly correct for Mg, but too high for K and Na. While the especially high increase in exchangeable Na can be accounted for by the addition of MARR, it should be noted that Na as a percentage of CEC in worm castings is >5% (i.e., 2.2 cmol<sup>+</sup>/kg in 51 cmol<sup>+</sup>/kg), meaning that Na in these worm castings before adding MARR was higher than the desired range of 0-1%. What impact, if any, these lopsided exchangeable cation ratios had on cotton production was outside the range of the present study.

Table 2 presents the total and leachable metal analyses for the first laboratory test. These data show that the addition of MARR increased the total concentrations of As, Cd, Cr, Fe, Mn, Ni and Pb in worm castings, although except for Cr, Mn and Pb, these were negligible. As MARR contains trace levels of Cr, Mn and Pb, an increase in concentrations was not unexpected. Data also show that raw worm castings are relatively high in Cr, Cu, Fe, Mn and Pb, however no concentration of metal in worm castings, either before or after MARR addition, was above the statutory limit for the application of compost in rehabilitation or catchment management (Department of Environment and Climate Change NSW, 2007).

 TABLE 2

 RESULTS OF TOTAL, TCLP AND WATER LEACHABLE METALS IN UNTREATED WORM CASTINGS AND IN A

 BLEND OF WORM CASTINGS AND MARR, AND TOTAL METALS IN SNL FROM THE FIRST LABORATORY TEST,

 INCLUDING ALLOWABLE TOTAL AND LEACHABLE METAL CONCENTRATIONS.

Parameter	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Total Metals (n	ng/kg)										
Worm Castings	7.4	0.7	23.9	188	39,000	0.2	567	25	107	1.0	0.5
Sub-sample	9.0	0.9	60.8	103	41,000	0.2	728	29	124	0.3	0.2
<i>Guideline</i> <sup>†</sup>	20	1.0	100	100		1.0	—	60	150	5.0	200
TCLPLeachab	le Metals	(mg/L)									
Worm Castings	0.07	0.001	0.01	0.38	2.2	< 0.001	4.9	0.02	0.24	0.005	1.8
Sub-sample	0.009	< 0.001	0.01	0.18	1.1	< 0.001	4.3	0.02	0.009	0.002	1.8
<i>Guideline<sup>‡</sup></i>	0.5	0.1	0.5			0.02	—	0.2	0.5	0.1	—
<i>Guideline</i> <sup>‡</sup>	5.0	1.0	5.0			0.2	—	2.0	5.0	1.0	—
Water Leachable Metals (mg/L)											
Worm Castings	0.01	< 0.001	0.002	0.02	0.003	0.01	< 0.001	0.004	< 0.001	0.05	0.58
Sub-sample	0.01	< 0.001	0.004	0.04	0.004	0.01	< 0.001	0.004	< 0.001	0.05	0.83
Supernatant Liquor Metals (mg/L)											
SNL	0.33	< 0.001	< 0.001	0.04	0.35	< 0.001	0.03	0.01	0.004	0.004	0.07

<sup>+</sup>Guideline for total metals in compost, Department of Environment and Climate Change NSW, 2007, p. 14; <sup>‡</sup>guideline for leachate from solids, including compost and other soil additives, Environment Protection Authority, 2009; and <sup>‡</sup>guideline for leachate from solid waste, Department of Environment, Climate Change and Water NSW, 2009, p. 17-18.

Table 2 also presents data on leachability of metals using both the TCLP and water leach methods. These data reveal the degree to which metals present in worm castings become plant available or leachable to the environment over a 20-year timeframe, and show that plant available TCLP metals decreased or remained the same as a result of MARR addition, consistent with previous findings on metals sequestration mechanisms (Fergusson, 2009). Water leached Cr, Fe and Zn increased slightly after MARR addition, but the leachability of all other metals remained the same. All leachable metals from

raw worm castings and worm castings combined with MARR were within acceptable guidelines for application as a fertiliser, with Table 2 presenting both the lower and high ends of allowable limits in Australia.

Total SNL metal concentrations from the first laboratory test are also presented in Table 2. These data represent the soluble metals fraction of worm castings, and shows that As, Fe, Mn, Ni and Pb were higher, Cd, Cu and Zn were the same, and Cr, Hg and Se were lower in the SNL than those leached from worm castings before and after MARR addition.

Table 3 presents the pH and microbial biomass data from the second laboratory test in Phase One. These data show that pH rose from 7.1 in raw worm castings to 8.2 after ViroGrow addition (due to the higher pH of both MARR and CaCO<sub>3</sub>), active bacterial content of the worm casting decreased from 492  $\mu$ g/ml to 399  $\mu$ g/ml (19%) and active fungi decreased from 76  $\mu$ g/ml to 38  $\mu$ g/ml (50%), however total bacteria increased from 93,696  $\mu$ g/ml to 130,560  $\mu$ g/ml (38%) and total fungi increased from 231  $\mu$ g/ml to 528  $\mu$ g/ml (130%).

#### TABLE 3

# PH, TOTAL AND ACTIVE BACTERIA, AND TOTAL AND ACTIVE FUNGI IN UNTREATED WORM CASTINGS AND IN A BLEND OF WORM CASTINGS AND MARR FROM THE SECOND LABORATORY TEST.

Parameter	рН	Total Bacteria (µg/ml)	% Change	Total Active Bacteria (µg/ml)	% Change	Total Fungi (µg/ml)	% Change	Total Active Fungi (µg/ml)	% Change
Worm Castings	7.1	93,696	—	492	—	231	—	76	—
Sub-sample	8.2	130,560	38↑	399	19↓	528	130↑	38	50↓

Of significance also was the anecdotal report by operators that plastic containers no longer "bloated" after MARR addition, possibly due to changes in pH, temperature and/or forms of oxygen present in the worm castings (i.e., hydroxides and oxyhydroxides versus carbon dioxide). From this data it can be concluded that worm castings with ViroGrow have higher than average total bacteria (130,560  $\mu$ g/ml versus 11,300  $\mu$ g/ml average, according to CIAAF [2012]), but lower than average total fungi (528  $\mu$ g/ml versus 25,300  $\mu$ g/ml), thus making the recommended fungi-to-bacteria ratio of 1:2.3 skewed heavily in favors of bacteria (i.e., 1:247).

The implications of this imbalanced ratio for cotton production, if any, are undocumented in the literature and in this study. However, these results do indicate that the Ca- and Si-based minerals in MARR may allow fungal oxalate secretions to solubilise the mineral Ca to form calcium oxalate crystals thus potentially being of value to soil and cotton production because the addition of ViroGrow apparently contributed to the significantly improved microbial biomass observed in this study. Together, the results of Phases One and Two answer research question 1).

#### 3.2 Phase Two

The results of the Phase Two field trial are presented in Table 4. Data indicate that the height of cotton plants and rate of growth over nine weeks was comparable between plants in the three paddocks, although early growth of plants in Paddock #2 was initially faster. Results also indicate that the number of plant nodes and the height:node ratios were essentially the same between paddocks, and the fruiting factors in all paddocks were comparable, although the fruiting factor in Paddock #1 was greater than either Paddocks #2 or #3, although the nodes above white fruit (i.e., plant nodes above mature bolls, which is a sign of cotton maturity, with less than  $\leq 5$  nodes a sign of plant maturity and time to harvest) were slightly higher in Paddock #3 than in Paddocks #1 or #2. Therefore, no significant difference on any measure could reasonably be inferred based on these data.

However, the most significant finding reported in Table 4 is yield. The data show that Paddock #1 had 7.77 bales/ha at harvest, while Paddocks #2 and #3 had 8.44 and 8.46 bales/ha respectively at harvest. [Note, a bale of Australian cotton weighs 227 kg, thus if the control produced 7.77 bales per ha, standard cotton farming practices produced 1,764 kg of cotton/ha during the trial; Australia's present cotton yield is closer to 2,270 kg/ha or 10 bales/ha (Weekly Times, 2015) although 15 bales/ha have been achieved, mostly due to the introduction of advanced biotechnical seed production, i.e., transgenic or genetically modified seed, which has also resulted in a reduction of insecticide use. Almost 100% of cotton grown in Australia is genetically modified (Agricultural Biotechnology Council of Australia, 2012)].

The difference in yields observed in this trial between the control, which was exposed to standard farming practices for the duration of the growing season, and the treatment paddocks, which used worm castings and ViroGrow, was 9%, a finding observed earlier in horticultural crops and pastures with and without the addition of N, P and K fertilisers by Summer *et al.* (1996b) and Summers *et al.* (2001). The picker driver also reported that bolls in Paddocks #2 and #3 were whiter and denser than in Paddock #1, and the basket appeared to fill up faster than it did in the control area. Phase Two therefore answers research question 2) in the affirmative.

Parameter	Paddock #1:	% Change	Paddock #2:	% Change	Paddock #3:	% Change					
Height											
Week 0	17.0	—	17.8	—	17.4	—					
Week 3	46	170↑	51.4	190↑	46.5	170↑					
Week 6	76.5	67↑	78	52↑	78	68↑					
Week 9	81.5	7↑	83.7	7↑	81.8	5↑					
Number of nodes											
Week 0	7.4	—	7.9	_	7.6	_					
Week 3	12.3	67↑	13.6	73↑	14.3	88↑					
Week 6	16	30↑	17	25↑	17	19↑					
Week 9	20	25↑	20	18↑	20	18↑					
		Height/r	node ratio								
Week 0	2.2	_	2.3		2.3						
Week 3	3.7	67↑	3.8	66↑	3.3	43↑					
Week 6	4.8	30↑	4.6	21↑	4.6	40↑					
Week 9	4.0	15↓	4.2	9↓	4.1	10↓					
Fruiting factor											
Week 0	0.96	—	1.1	_	0.7	_					
Week 9	13.7	1,320↑	12.9	1,070↑	11.1	1,480↑					
Nodes above white fruit in Week 9)	1.87	—	1.33	_	1.93	_					
Yield (bales/ha) in Week 9	7.77	_	8.44	_	8.46	_					

TABLE 4COTTON GROWTH PARAMETERS FOR PADDOCKS #1, #2 AND #3.

## IV. CONCLUSION

Phase one of this research provides evidence of the chemical and biological qualities of fertiliser when MARR is added to worm castings. Beneficial water soluble cations Ca, K and Mg were somewhat improved, but Na was significantly increased as a result of MARR addition. The ratio of cations in both worm castings and worm castings with MARR is not ideal, however; what impact, if any, such a ratio would have on soil matrices in cotton farming, and on agriculture in general, remains an unanswered question.

Total metals and water leachable metals generally increased but acid leachable metals either remained the same or declined as a result of MARR addition. Similarly, total bacterial and fungal biomass increased significantly as a result of MARR addition, although active bacteria and fungi decreased as a percentage of total microbial biomass. The implications of these chemical and biological changes were not the detailed subject of this paper, and further research on the relationship of worm castings and MARR remains to be conducted, although the possible relationship between fungal oxalate secretions and the formation of calcium oxalate crystals has been somewhat advanced.

This research program aimed at demonstrating whether worm castings amended with MARR could benefit cotton production, which the research attempted to answer in Phase Two. The two fertiliser types described herein were expected to benefit the crop via a number of possible biochemical mechanisms, including improving the uptake of nutrients by the plant, reducing the number of disease incidents, improving soil water retention, reducing soil compaction, and positively changing soil pH. Which (if any or in what combination) of these mechanisms caused the observed improved cotton yields reported in Table 4 was not isolated by this research design, but the difference between non-worm castings fertiliser and fertilisers developed by blending worm castings with MARR is notable.

This research design similarly did not investigate if or to what degree Fertilisers #1 and #2 could supplement or replace standard fertiliser applications; all paddocks received standard applications but only Paddocks #1 and #2 received fertilisers derived from worm castings, begging the question whether it was a combination of standard fertilisers plus worm castings and ViroGrow which caused the observed difference in yields or whether the worm castings fertilisers could themselves have produced comparable yields with reduced or different standard fertiliser applications. Moreover, the design did not identify to what degree MARR played a part in this observed yield; for example, would lower or even no additions of MARR in worm castings have resulted in the same outcome?

Fertilisers #1 and #2 were applied to Paddocks #2 and #3 by spraying at a time when plant nodes averaged 7.6. Further research on the use of worm casting fertilisers applied by injection or truck implant should be carried out to determine if these alternate methods would deliver nutrients more directly to plant root systems, although injections and truck implants are usually only recommended when spraying or drenching are not effective, which was not the case in this trial.

Similarly, only standard measurements of plant height, number of nodes, fruit counts, number of flowers, and nodes above white flower were carried out in this experiment, but seedling emergence, seedling health and plant stand numbers could also have been included in the study design to provide a richer profile of growth patterns. Further research on the "carried over" benefits of applying modified worm castings would also constitute a worthy direction for further investigation; if worm castings and MARR over time continue to have an impact on the life-cycle of soil, such benefit should increase productivity and yields, however the sustainable use of these novel fertilisers was beyond the scope of this investigation.

Nevertheless, this study does provide enough preliminary evidence to suggest that the addition of 10% MARR to worm castings does improve yields in cotton production.

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